range of members of the myosin family. In recent work, we have combined this approach with the use of small molecule effectors of myosin motor activity. We identified pentabromopseudilin (PBP) and related halogenated alkaloids as potent inhibitors of myosin-dependent processes such as isometric tension development, unloaded shortening velocity, and in vitro motility. Coupling between the actin and nucleotide binding sites is reduced in the presence of these inhibitors. PBP-induced changes in rate constants for ATP-binding, ATP-hydrolysis and ADP dissociation extend the time required per actin-activated myosin ATPase cycle. Additionally, the ratio of time spent per ATPase cycle in strong and weak binding states is shifted by PBP and related compounds in favor of non-force generating states. To elucidate the binding mode of these compounds, we crystallized their complexes formed with the Dictyostelium myosin-2 motor domain. In every case, the electron density for the small molecule inhibitor is unambiguous. All compounds bind to a novel allosteric site near actin-binding residues at the tip of the 50-kDa domain. The residues involved in the binding of this new class of inhibitors are only moderately conserved between the members of the different myosin classes. This is consistent with the observed differences in IC50 values. The results of molecular modeling studies show that these isoform-specific variations in the extent of inhibition can be predicted at least in trend for each of the new compounds.

2849-Symp
The collective mechanics of myosin in muscle
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2850-Symp
An Integrative Analysis of the Muscle Myosin Motor Using Genetic and Transgenic Tools
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We use *Drosophila melanogaster* to investigate the mechanisms by which myosin heavy chain isoforms contribute to muscle-specific cyto-architectures and contractile properties. We expressed various myosin isoforms in transgenic indirect flight muscles. Along with our collaborators, we examined 1) ATPase, *in vitro* actin sliding, step size and structure of the isolated myosin, 2) ultrastructure and mechanical properties of muscle fibers, and 3) locomotory abilities of the transgenic organisms. We found that isoform-specific differences in myosin cause relatively small structural variations in muscle assembly, but are critical to myofibril stability and function. ATPase, *in vitro* motility and fiber mechanical assays show that embryonic and indirect flight muscle isoforms represent slower and faster myosins, respectively. We have used a series of chimeric transgenes to study the function of the alternative domains that vary among the *Drosophila* myosins. We found that the converter is a key determinant of isoform-specific properties, and that some mechanisms of fine tuning myosin function differ from those of vertebrates.
Drosophila point mutants also help define the function of structural domains of myosin. We analyzed point mutations in the transducer domain near the nucleotide-binding pocket. While increased myosin ATPase and actin sliding ability of one transducer mutation induce dramatic degeneration of the indirect flight muscles, another mutation that reduces these properties does not affect myofibril stability. The effects of these mutations on the *Drosophila* heart are disparate as well, with increased function yielding restrictive cardiomyopathy and decreased function leading to dilated cardiomyopathy. These phenotypes parallel those arising from myosin mutations in humans. *Drosophila* may thus serve as a useful model for studying the molecular basis of cardiomyopathy, as well as mechanisms of its suppression.

Platform AX: Protein Dynamics II

2851-Plat
Slow Correlated Movement of Structural Elements in Hemoglobin and Myoglobin
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Structural fluctuations of proteins in solution are often reflections of functional movements and appear to follow low energy pathways that have evolved to accommodate conformational changes triggered, for instance, by binding to ligands or catalysis of reactions. The study of these fluctuations can provide substantial insight into protein function. The spatial extent and dynamics of intramolecular movements in proteins are highly dependent on the environment of the protein, being particularly sensitive to the concentration of proteins and other constituents in the solution. Consequently, studies as a function of protein concentration may provide further insight into the nature of these fluctuations than is possible when experiments are carried out at a single protein concentration. Wide-angle x-ray solution scattering (WAXS) and neutron spin echo spectroscopy (NSE) provide substantial information about the spatial extent and time scale, respectively, of structural fluctuations in solution. They are particularly well suited to the study of slow, correlated movements that are central to many protein functions, providing information complementary to that obtainable by x-ray crystallography and NMR. Using these approaches, we have characterized the spatial and temporal properties of hemoglobin and myoglobin across a wide range of environments. For these proteins, the spatial extent and dynamics of correlated movements both increase rapidly as protein concentration decreases below about 50 mg/ml. A combined analysis of WAXS and NSE data indicates that the slow correlated fluctuations in these molecules are dominated by movement of relatively rigid alpha helices within the subunits. These fluctuations are highly dependent on the ligation state of the molecules and are altered by mutations that impact the function of the molecules.

2852-Plat
Method to Characterize the Global Dynamics of Proteins by Temperature Dependent Phosphorescence Lifetime Measurements. Application for the Allosteric Effect in Hemoglobin
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At the glass transition temperature of proteins, large scale, anharmonic motions of the conformation become activated. It has been shown that in this temperature range the phosphorescence lifetime of Trp residues that is long (several s) at cryogenic temperatures become efficiently quenched. We report the elaboration of a systematic method based on this observation with the purpose to use this effect for characterizing the global conformational dynamics of proteins. In this method, special conditions were determined under which the onset temperature of phosphorescence quenching can be clearly separated from the slaving effect of fluctuations activated in the solvent, and the relevance of this temperature for the global dynamics of the protein has been verified by parallel experiments on human alpha-oxyFe-betaZn-Hemoglobin, w.t. horse heart Myoglobin and Trp mutant forms of a two-domain protein, yeast phosphoglycerate kinase. The method was used to characterize the role of global dynamics in the allosteric response of human hemoglobin to the binding of effectors as Cl, IHP, DPG, BZF. The quenching effect showed a two state behavior with a characteristic onset temperature sensitive to the presence and quality of allosteric effectors. The data points were fit by a simple two state model and the thermodynamic parameters - enthalpy and entropy changes characteristic for the quenching effect were discussed in terms of oxygen hopping models. The study on hemoglobin verifies the concept that in allosteric regulation, the global dynamics of the protein tetramer plays important role.
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2853-Plat
Computational Methods for Predicting Sites of Functionally Important Dynamics
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Understanding and controlling biological function of proteins at the atomic level is of great importance; allosteric mechanisms provide such an interface. Experimental and computational methods have been developed to search for residue mutations that produce changes in function by altering sites of correlated motion. These methods are often observational in that altered motions are achieved by random sampling without revealing the underlying mechanism(s). We present two deterministic methods founded on structure-function relationships that predict dynamic control sites (i.e. locations that experience correlated motions as a result of altered dynamics).
The first method (‘‘static’’) is based on a single structure conformation (e.g. the wild type (WT)) and utilizes a graph description of atomic connectivity. The local atomic interactions are used to compute the propagation of contact paths. This description of structure connectivity reveals flexible locations that are susceptible to altered dynamics.
The second method (‘‘dynamic’’) is a comparative analysis between the normal modes of a WT structure and a mutant structure. A mapping function is defined that quantifies the significance of the motions in one structure projected onto the motions of the other. Each mode is considered up- or down-regulated according to its change in relative significance. This description of altered dynamics is the basis for a motion correlation analysis, from which the dynamic control sites are readily identified.